Pestic. Sci. 1997, **51**, 7–20

Persistence of Tebufenozide in Aquatic Ecosystems under Laboratory and Field Conditions

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(Received 7 March 1996; revised version received 14 January 1997; accepted 3 April 1997)

Abstract: The persistence and dissipation behaviour of tebufenozide, an ecdysone agonist, were investigated: (1) under laboratory conditions in aquatic models set up in glass aquaria, and (2) under field conditions in in-situ aquatic enclosures deployed in a mixed-wood boreal forest lake.

Two models were set up in the laboratory study (Study I), which was conducted at constant conditions of temperature, water pH and photoperiod. In Model I, partitioning of tebufenozide from sediment, treated at a concentration of 1400 μg kg^{-1}, into untreated water was examined. The results showed that the chemical moved very little from the treated sediment into water. The concentration in sediment and water decreased gradually during the 90-day incubation period. Tebufenozide disappeared faster from the top layer of sediment than from the middle and bottom layers. The half-lives of disappearance were 64 days for the top layer but >90 days for the middle and bottom layers respectively. In Model II, partitioning from water, treated at a concentration of 350 μg litre^{-1}, into untreated sediment was investigated. The results showed that the chemical moved from treated water into sediment due to adsorption. Little vertical downward movement of the adsorbed residues from the top layer of sediment occurred into layers beneath. The adsorbed residues were also not released readily back into water. The concentration in water and sediment decreased gradually during the 90-day incubation period. The half-life of dissipation from water was 67 days.

The field microcosm study (Study II), conducted under fluctuating conditions of temperature, water pH and photoperiod, involved application of tebufenozide onto aquatic enclosures at four concentrations of 0·05, 0·10, 0·26 and 0·5 mg litre^{-1}. This study also showed that the chemical moved downwards from the applied location and was adsorbed onto sediment. The chemical persisted longer in Study II than in Study I. Tebufenozide, being photo-labile, probably degraded faster after constant exposure to light in Study I than after exposure to fluctuating light in Study II. At 90 days after treatment in Study I, only about 55% of the applied material persisted in the sediment, and there was little accumulation. In Study II, the material not only persisted but also was accumulated in the sediment, since at 92 days post-treatment the residues were about 25 times higher than the applied concentration level. Residues in water also decreased more rapidly in Study I than in Study II, because the concentration at 90 days post-treatment was about 41% of the applied value. In Study II, however, about 65% of the applied chemical persisted in water at 92 days post-treatment. While the long persistence of tebufenozide in both the laboratory and field studies was attributable to its low vapour pressure, low water solubility, high octanol/water partition coefficient etc., the differences in the persistence characteristics observed in the two studies were due to the fluctuating environmental conditions and water pH encountered in the field study, compared with the constant environmental conditions and water pH utilized in the laboratory study.

Pestic Sci., **51**, 7–20, 1997
No. of Figures: 1. No. of Tables: 10. No. of Refs: 12

Key words: aquatic enclosures, dissipation behaviour, adsorption to sediment, vertical mobility, tebufenozide

 Pestic. Sci. 0031-613X/97/$17.50. Printed in Great Britain

1 INTRODUCTION

Tebufenozide, [*N-tert*-butyl-*N'*-(4-ethylbenzoyl)-3,5-dimethylbenzohydrazide; 'Mimic', RH-5992] is a novel type of insect growth regulator interfering with the moulting process of lepidopteran insects.[1,2] It acts as an agonist or mimic of insect moulting hormone, 20-hydroxyecdysone, causing feeding inhibition, premature ecdysis and eventual death of the exposed insects. The material is appreciably active against the eastern spruce budworm, *Choristoneura fumiferana* Clemens,[3] a destructive defoliator that causes considerable damage to the spruce/fir forests of eastern North America, but it shows low mammalian, avian, fish and *Daphnia* toxicity in standard tests.[4] These properties suggest that this material may show a relatively narrow spectrum of activity and become an alternative to broad-spectrum chemical insecticides in forest pest management.

Aerial field trials conducted recently by researchers in Canadian Forest Service have shown that tebufenozide is effective in controlling the eastern spruce budworm (Cadogan, pers. comm.). Before an insecticide can be used operationally in Canada for forest insect control, it must be registered under the Pest Control Products (PCP) Act. One of the prerequisites of the PCP Act is that the chemical should not have any adverse effect on the environment, or on human health. Therefore, a comprehensive data package is required on the environmental behaviour of tebufenozide before the chemical can be registered. In addition, information on the combined influence of the physicochemical properties of the compound and of environmental substrates on fate and persistence is valuable to understand the possible short- and long-term adverse effects on the environment.[5]

At present, literature information is sparse on the environmental chemistry and behaviour of tebufenozide in aquatic substrates under Canadian use conditions. Therefore, a series of laboratory and field microcosm studies were undertaken to investigate the mobility, accumulation and dissipation patterns of tebufenozide in water, suspended solids and sediment samples after application onto aquatic ecosystems under controlled conditions.

2 EXPERIMENTAL METHODS

Two studies were conducted, Study I under controlled conditions in the laboratory, and Study II under field conditions in aquatic enclosures. Study I involved two aquatic models: in Model I the partitioning of tebufenozide from treated sediment into untreated water was examined, and in Model II the movement from treated water into untreated sediment was investigated.

2.1 Study I: Behaviour of tebufenozide in aquatic models under laboratory conditions

The study was conducted during the spring/summer months of 1992. Sediment samples were collected from a lake (Greenwater Lake, *c.*3·0 ha in area), located about 80 km northwest (46°53′N, 84°03′W) of Sault Ste. Marie, Ontario, Canada. Details of the organic matter content and texture of the sediment are given in Table 1. The samples were transferred to a large, clean plastic container, stored immediately at 0°C on ice and brought to the laboratory. In the laboratory, the samples were passed through a 2-mm sieve to remove debris, small stones etc. and filtered under suction using a Whatman No. 1 paper to remove the adsorbed water. Aliquots of the sediment (average moisture content 57%, $n = 126$) were extracted with dichloromethane, solvent-partitioned, concentrated, subjected to column clean-up and analyzed for tebufenozide, using the high-performance liquid chromatographic (HPLC) method of Sundaram *et al.*[6] This was done to determine whether any inadvertent contamination of the lake had occurred from spray drift during the previous year's operational programs. Aliquots of the wet sediment were treated with an acetone solution of tebufenozide (5 mg ml^{-1}) (>98% pure). The sediment samples were taken in glass-stoppered conical flasks and agitated horizontally in a mechanical shaker for 2 h to facilitate uniform distribution of the chemical in the sediment and to provide an initial concentration of 1400 μg kg^{-1} wet weight.

Model I consisted of a glass aquarium tank (36 × 24 × 48 cm). Forty plastic vials (3·2 cm ID × 7·0 cm high, weight 13·3 g, 50 ml capacity), each filled with 40 g (wet weight) of the treated sediment and attached to Teflon straps for easy retrieval, were laid out at the bottom. The aquarium was then carefully filled with 40 litres of the lake water (pH, 6·7) (analyzed previously for tebufenozide to determine if there would be any background contamination). Model II was set up similarly, except that the plastic vials contained untreated sediment samples. After setting for 24 h, the water of Model II was fortified with tebufenozide (5 mg ml^{-1} in acetone) to give an initial concentration of 350 μg $litre^{-1}$. The control model consisted of untreated sediment and water, set up in a similar fashion. Another set of replicate aquaria was established, so that samples with low residues could be pooled to provide measurable concentrations. All aquaria were held for incubation at 15°C in an environmental chamber for 90 days. To provide light in the chamber, two 400-W multivapour discharge lamps were used continuously throughout the 90-day incubation period. The average intensity and wavelength range of the lamps were measured close to the aquaria (683 W m^{-2} and 320–1100 nm respectively), and were found to be similar to the values (678 W m^{-2} and 320–

TABLE 1
Study II: Site Description, Water Chemistry and Sediment Texture

Characteristics	*Description*
Location	Greenwater Lake, northern Ontario, Canada
Sediment (0–15 cm depth)	Organic, flocculent [Organic matter 28·2%, sand 10%, silt 55%, clay 35% cation exchange 36 meq 100 g^{-1}, specific surface area 180 m^2 g^{-1}, density (dry wt) 1·31 g ml^{-1}] [density of surficial sediment (dry wt) 0·48 g ml^{-1}]
Depth (average) of in-situ heavy duty polyethylene enclosures (m)	4·0
Average area of enclosures (adjusted) (m^2)	25
Average volume of water in enclosures (adjusted) (litre)	100 000
Water pH (range)	6·4–7·6
Turbidity (JTU) (range)	4·9–16·4
Specific conductance (μmhos cm^{-1}) (range)	14·3–16·2
Hardness (mg $CaCO_3$ $litre^{-1}$) (range)	8·8–11·7
Dissolved oxygen (mg $litre^{-1}$) (range)	9·8–11·2
Total nitrogen (mg $litre^{-1}$) (range)	0·59–1·12
Total phosphorus (mg $litre^{-1}$) (range)	0·02–0·04
Water temperature (°C) (range)	
June	12·0–18·5
July	11·2–19·3
August	14·8–19·5
September	10·7–16·7
October	5·2–10·6
November	2·1–4·0 (18 Nov., gradual freeze-up of lake)
Study initiated	17 June 1992 (0 day)
Terminated for winter months	18 Nov. 1992 (154th day)
Sampling dates after snow-melt in spring/summer of 1993	25 May 1993 (342nd day), 7 June 1993 (355th day) and 15 July 1993 (393rd day)

1100 nm) of the natural sunlight measured near the aquatic enclosures used in the field study. The exterior of the aquarium was covered with aluminum foil from the bottom to a height of about 10 cm to prevent light from falling on the sediment from the sides. At various intervals during incubation (0, 1, 2, 4, 7, 10, 15, 20, 25, 30, 35, 45, 55, 70 and 90 days after tebufenozide treatment), a 40-ml sample of water (0 to 10 cm from the top surface) and two plastic vials containing the flooded sediment were carefully retrieved from each aquarium for the analysis[6] of tebufenozide. Prior to the start of this investigation, test trials were run to determine whether the sampling depth would make a difference in water concentration levels of tebufenozide. Analysis showed little difference in concentrations between samples collected at various depths, thus indicating uniform distribution of tebufenozide in water. Samples of the sediment in vials were frozen and sliced into 2-cm (top), 2-cm (middle) and 3-cm (bottom) layers. The corresponding layers from the duplicate vials from each of the two replicate aquaria were pooled, thawed and filtered under suction to remove the adsorbed water as much as possible. The wet sediment samples were then analyzed[6] for tebufenozide to determine the average vertical distribution in the sediment. The pH of water was measured at every sampling interval to determine if the pH changed during the 90-day period.

2.2 Study II: Behaviour of tebufenozide in in-situ enclosures under field conditions

2.2.1 Site description and weather conditions

The study was conducted in 1992 in the Greenwater Lake. Details of the aquatic enclosures, water chemistry (monitored throughout the course of the study), and sediment texture are given in Table 1. The climate around the lake area during the months of June to November in 1992 (when the study was conducted) was cool, cloudy and wet. The monthly rainfall (cm) during this period was June 10·9, July 9·8, August 9·3, September 12·5, October 9·1 and November 7·4, to

provide a total of 59 cm. The winter was long and relatively severe, with temperatures frequently falling below −20°C. The total snowfall during the winter months of December 1992 to April 1993 amounted to about 110 cm.

2.2.2 In-situ enclosures

Details of the construction and design of the in-situ enclosures are described elsewhere,[7,8] and a brief sketch is given in Fig. 1. Four enclosures, each 5 × 5 m square, were constructed using impervious heavy duty polyethylene sheets. They were deployed individually in a shallow bay (mean depth 4·3 m) of the lake, where the bottom slope was minimal. The enclosures were anchored firmly in place, using the wooden float on the top and the angle iron at the bottom, four to five weeks prior to tebufenozide treatment so that the water inside the enclosures would attain equilibrium with that of the lake. The lower part of the enclosure was tucked under the sediment and held firmly to the bottom of the lake using the sediment layer as a sealer to prevent movement of the applied tebufenozide to the lake water outside. The water level in each enclosure was monitored throughout the investigation, and the mean (±SD) volume of water in each enclosure was 1·0 (±0·1) × 10^5 litres (Table 1).

2.2.3 Application of tebufenozide

A tebufenozide 240 g litre^{-1} suspension concentrate (RH-5992 2F; Rohm and Haas), was diluted with water and mixed well by vigorous agitation. Required volumes, corresponding to 700, 360, 140 and 70 g AI ha^{-1} (with the operational rates ranging from 70 to 140 g AI ha^{-1}),[9] or nominally 0·50, 0·26, 0·10 and 0·05 mg AI per litre of water, were carefully applied onto the four respective enclosures, using a backpack sprayer (Model 4F, R&D Sprayers, Inc., Opelousas, LA) equipped with a 2-m long hand-held boom with an open-orifice nozzle, pressurized by carbon dioxide at 210 kPa. The nozzle was held approximately 30 cm above the water surface, and spray was applied in three replicates to achieve uniform distribution of tebufenozide over the entire area. Each enclosure was equipped with a 40-cm high wall of plastic sheeting around it to prevent contamination of the neighbouring enclosures by drift.

2.2.4 Distribution of tebufenozide in surface microlayer

To determine surface microlayer concentrations of tebufenozide, glass fibre filter discs (GFF discs, 3·7 cm diameter, PN #66214, Gelman Sciences, Ann Arbor, MI 48106, USA) were laid out flat (one at each corner of the enclosure per sampling interval) on the water surface using a pair of forceps, and were taken out after 5 s. In this manner, two replicate samples were collected (8 GFF discs) per sampling interval. The excess water was allowed to drip, and the four GFF discs corresponding to each replicate sample were pooled, placed in glass vials (30 ml capacity) and stored in coolers containing ice (0°C). Sampling was done at 1·0 h pre-spray, and at 1·0, 4·0, 8·0 and 24 h post-spray.

2.2.5 Vertical mobility of tebufenozide in water

Vertical mobility of the chemical was studied only in two enclosures which were treated with 0·05 and 0·26 mg litre^{-1} concentration levels. Water samples were taken from three specific depths (0·5, 1·5 and 2·5 m from the water surface) at the same intervals of time as those used for sampling the surface microlayer. Two replicate samples (each 1·3 litre) were collected using the Kemmerer Water Sampler (Model 1200 B10; Wildco, Saginaw, MI 48602, USA). Each sample was stored separately in amber-coloured glass bottles (2 litre capacity) at 0°C.

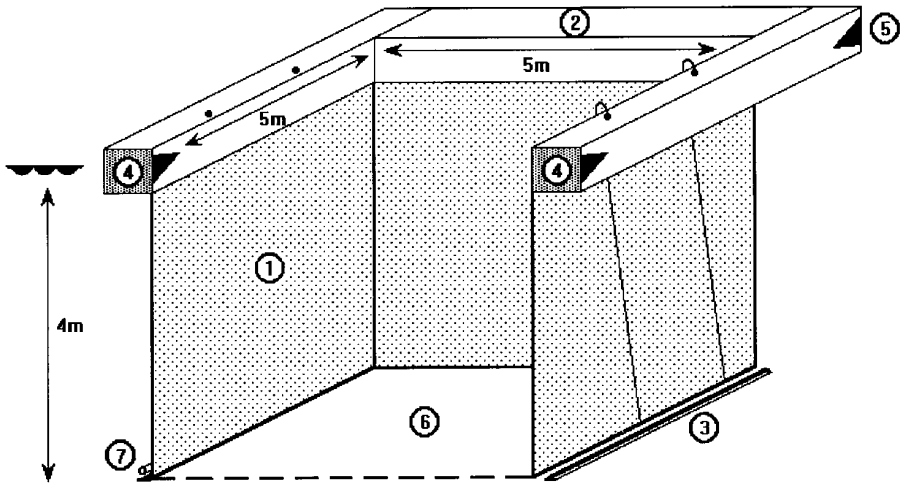


Fig. 1. Design details of in-situ enclosures: (1) 6-mm polyethylene liner; (2) 17·8 × 35·6-cm wooden float; (3) 2·5 × 2·5 × 0·32-cm angle iron; (4) polyethylene-encased styrofoam flotation; (5) 15·2-cm leaf hinge with clevis pin; (6) natural sediment bottom; (7) 0·32-cm diameter elastic shock cord.

2.2.6 Persistence of tebufenozide in water
Water was sampled from each enclosure at 3 h pre-spray, and at 0·33, 1, 2, 3, 5, 8, 12, 16, 21, 28, 35, 49, 70, 92, 119 and 154 days post-spray until 18 Nov. 1992, when the water started to freeze. Further sampling was done after the snow-melt during the spring months in 1993. These sampling days corresponded to 342, 355 and 393 days (15 July 1993) after spray, and sampling was terminated afterwards. Water samples were collected as a 3·0-m deep water column by using an integrated sampler (25 mm OD × 3·7 m long aluminum tube fitted at one end with a PVC check valve). Four integrated samples were taken from each enclosure at each interval after spray, two each of the four samples were pooled into a clean glass bottle (4-litre capacity) to provide two composite samples in total, and capped. After mixing thoroughly, two replicate samples, each 1·3 litre, were collected from each composite sample, transferred into a 2-litre amber-coloured glass bottle, and stored at 0°C.

2.2.7 Tebufenozide residues in suspended solids
To determine the amount of tebufenozide associated with suspended solids, water samples were collected (as described above) only from the two enclosures which received 0·26 and 0·50 mg litre^{-1} concentration levels. Two of the four samples were pooled to provide two composite samples for analysis, placed in 2-litre amber-coloured glass bottles and stored at 0°C.

2.2.8 Persistence of tebufenozide in sediment
Sediment samples were collected at the same time intervals as those used for water. At each interval, a single sediment core was taken (twice to provide two samples) from each enclosure by gently lowering the Wildco Stratification Core Sampler (Hoskin, Ontario) to the bottom of the lake and allowing it to sink into the sediment to a depth of 15 cm, and then pulling it out carefully without disturbing the surroundings. Each sample was transferred to a clean mason jar (500 ml capacity) and stored at 0°C.

All samples were transported to the analytical laboratory in Sault Ste. Marie, and stored frozen at −20°C until analyzed.

2.2.9 Extraction and analysis
Prior to the start of the entire investigation, radio-tracer studies were conducted using [^{14}C]tebufenozide to determine adsorption to different surfaces; the data showed negligible adsorption of the chemical to polyethylene, glass and Teflon surfaces.[9]

Analytical grade tebufenozide (purity >99·6% w/w) was provided by Rohm and Haas Company. The water samples were processed as described by Sundaram.[10] To remove the suspended solids from water, the samples were filtered under suction using PTFE™ membrane filters (5 μm pore, PN #P4H047, Gelman Sciences, Ann Arbor, MI 48106, USA). The sediment samples were passed through a 2-mm sieve to remove debris, small stones etc. and filtered under suction using Whatman No. 1 filter paper to remove the adsorbed water prior to analysis. Tebufenozide residues present in aliquots of the substrates were extracted and analyzed using the HPLC method reported by Sundaram *et al.*[6]

3 RESULTS AND DISCUSSION

3.1 Study I: Behaviour of tebufenozide in aquatic models under laboratory conditions

3.1.1 Model I: Disappearance of tebufenozide from the sediment, movement into water, and vertical distribution
The concentrations of tebufenozide in the top, middle and bottom layers of the flooded sediment of Model I are given in Table 2, along with the levels that moved into water at different intervals of incubation. Pre-spray and control samples of sediment and water did not contain any tebufenozide, and no change in water pH was noted during the 90-day incubation. The amount of tebufenozide that moved into water appears to have come more from the top 2-cm layer of the sediment than from the other two layers. This is probably the reason why the concentration of the chemical decreased more rapidly from the top layer than from the other two layers. The top layer was also more exposed to light, and, therefore photodegradation of tebufenozide could have occurred more in the top layer than in the other two layers. In a controlled laboratory study,[11] tebufenozide was shown to undergo photodegradation in water when exposed to sunlight. Among the three layers, disappearance of residues was the slowest from the bottom 3-cm layer of the sediment. However, the residue levels in all the three layers were still appreciable even at 90 days after treatment (Table 2), thus indicating an overall slow rate of disappearance. In contrast, the residue level (μg litre^{-1}) in water increased gradually from 0·096 on the first day, reached a maximum of 0·488 on the seventh day, and declined afterwards to 0·084 on the 90th day.

The concentrations of the residues in the three sediment layers were subjected to regression analysis, and were found to fit well into the following equations:

$$Y = B\,\mathrm{e}^{-Ct} \tag{1}$$

$$\mathrm{Log}_{10}\,Y = \mathrm{Log}_{10}\,B - (C/2.303)t \tag{2}$$

$$\mathrm{DT}_{50} = 2.303\,\mathrm{log}_{10}\,2/C \tag{3}$$

where Y = residue concentration at time t, B = the theoretical initial residue level, C = the rate constant of the exponential decline of residues, and DT_{50}, the half-life (the time required for 50% of the initial residue to disappear) of persistence. Values of the rate constant, C,

TABLE 2
Study I—Model I: Disappearance of Tebufenozide from Sediment and Movement into Water at 15°C in an Environmental Chamber

	Residue levels of tebufenozide							
Incubation time (days)	*Sediment (top 2 cm) ($\mu g\ kg^{-1}$) (μg)*[a]	*Sediment (middle 2 cm) ($\mu g\ kg^{-1}$) (μg)*[a]	*Sediment (bottom 3 cm) ($\mu g\ kg^{-1}$) (μg)*[a]	*Total residue in sediment*[b] *(μg)*	*Water at 0–10 cm from the top ($\mu g\ litre^{-1}$)*	*Total amount in water (μg)*	*Total residue (μg)*[c]	*Total residue (%)*
0	1400 (15·96)	1400 (15·96)	1400 (24·08)	2240 (56·00)	ND	ND	2240	100
1	1357 (15·47)	1385 (15·79)	1375 (23·65)	2196 (54·91)	0·096	3·84	2200	98·2
2	1281 (14·60)	1322 (15·07)	1363 (23·44)	2124 (53·11)	0·288	11·52	2136	95·4
4	1199 (13·66)	1271 (14·49)	1328 (22·85)	2040 (51·00)	0·410	16·40	2056	91·8
7	1134 (12·93)	1220 (13·91)	1293 (22·24)	1963 (49·08)	0·488	19·52	1983	88·5
10	1068 (12·18)	1172 (13·36)	1273 (21·90)	1898 (47·44)	0·332	13·28	1911	85·3
15	1008 (11·50)	1125 (12·83)	1257 (21·63)	1838 (45·96)	0·206	8·24	1846	82·4
20	921 (10·50)	1070 (12·20)	1221 (21·00)	1748 (43·70)	0·202	8·08	1756	78·4
25	875 (9·98)	1022 (11·65)	1202 (20·67)	1692 (42·30)	0·214	8·56	1701	75·9
30	817 (9·31)	985 (11·23)	1173 (20·18)	1629 (40·72)	0·208	8·32	1637	73·1
35	763 (8·70)	943 (10·75)	1144 (19·68)	1565 (39·13)	0·166	6·64	1572	70·2
45	696 (7·93)	895 (10·20)	1120 (19·27)	1496 (37·40)	0·156	6·24	1502	67·1
55	648 (7·39)	842 (9·60)	1085 (18·66)	1426 (35·65)	0·130	5·20	1431	63·9
70	576 (6·57)	801 (9·13)	1052 (18·09)	1352 (33·79)	0·122	4·88	1357	60·6
90	518 (5·91)	763 (8·70)	1032 (17·76)	1295 (32·37)	0·084	3·36	1298	57·9

[a] Values in parentheses refer to the actual amount of tebufenozide present in the individual layers of the sediment in one plastic vial.
[b] Values of the total residue refer to the entire amount present in all of the 40 plastic vials placed in one glass tank. These were obtained from the total amount present in one plastic vial (given in parentheses) by multiplying the values by 40.
[c] Values in this column refer to the sum of total residues in 40 plastic vials and the total concentration levels in 40 litres of water.

coefficient of determination (R^2), and DT_{50} are given Table 3 (if the calculated DT_{50} was greater than the 90-day duration of the experiment, the values were then reported as >90 days). The actual amount of tebufenozide (μg) in each of the three sediment layers was calculated from the concentrations ($\mu g\ kg^{-1}$) measured, added together, and multiplied by the total number of vials (40) to compute the entire amount present in the sediment in each tank (Table 2). Similarly, the total amount of tebufenozide (μg) in the 40 litres of water in each tank was calculated from the concentrations measured ($\mu g\ litre^{-1}$). The total residues in the sediment and water were added together to obtain the total residue (μg) in the entire tank (Table 2). The two sets of data (total residue in sediment, and total residue in water) were fitted into eqns (1) to (3) to calculate the DT_{50} (Table 3). The DT_{50} for the top layer was 64 days, and for the middle and bottom layers >90 days. Thus the rate of disappearance was lower for the middle and bottom layers than for the top layer.

3.1.2 Model II: Disappearance of tebufenozide from water, movement into sediment and vertical distribution

The concentrations of tebufenozide in water samples of Model II are given in Table 4 at different intervals during the 90-day incubation period, together with those in the top, middle and bottom layers of the sediment. The residues in water ($\mu g\ litre^{-1}$) showed a gradual decline from 350 on day zero to 145 on the 90th day. In contrast, the concentration ($\mu g\ kg^{-1}$) in the top layer of sediment increased gradually from 873 on the first day to 3444 on the 15th day, and declined afterwards to 1656 on the 90th day. The residue in the middle layer showed a similar initial increase, followed by a gradual decrease, although the concentration level was much lower than in the top layer. No residue was detected in the bottom layer (Table 4), thus indicating little downward movement of tebufenozide from the top to the bottom layer. The chemical appears to have been adsorbed to the sediment when water came first into contact with the top layer. However, the top layer was more exposed to light than the other two layers, and one would expect appreciable photodegradation in the top layer compared to that in the other two layers. The fact that residue levels in the top layer were much higher than in the other two layers indicates that adsorption played a relatively more important role in the build-up of residues than photodegradation. Furthermore, there were no organisms moving around in the sediment to facilitate physical mixing of the different sediment layers. The residues in water and in sediment were still measurable even at 90 days after treatment

TABLE 3
Study I: Dissipation Kinetics of Tebufenozide in Sediment and Water in Aquatic Models I and II Using the Equations

$$Y = Be^{-Ct}$$
$$\mathrm{Log}_{10} Y = \mathrm{Log}_{10} B - (C/2{\cdot}303)t$$
$$\mathrm{DT}_{50} = (2{\cdot}303 \log_{10} 2)/C$$

Samples analyzed	*Rate constant C*	*Coefficient of determination (R^2)*	*DT_{50} (days)*
		Model I	
Sediment (top 2 cm)[a]	−0·0108	0·947	64
Sediment (middle 2 cm)[a]	−0·0067	0·922	>90
Sediment (bottom 3 cm)[a]	−0·0033	0·929	>90
Total residue in the sediment[b]	−0·0065	0·926	>90
Total residue in the glass tank[c]	−0·0060	0·928	>90
		Model II	
Total residue in the water[d]	−0·0104	0·898	67
Total residue in the glass tank[c]	−0·0096	0·924	72

[a] Values of Y used in the exponential equation were the concentrations (μg kg^{-1}) of tebufenozide in individual layers of the sediment.
[b] Values of Y represent the total residue present (μg) in sediment in 40 plastic vials placed in the aquarium tank.
[c] Values of Y represent the sum of total residue (μg) in sediment in 40 plastic vials and total residue (μg) in 40 litres of water.
[d] Values of Y represent the total residue (μg) present in 40 litres of water in the aquarium tank.

(Table 4), thus indicating an overall slow rate of disappearance.

The total amount of tebufenozide (μg) in the 40 litres of water in each tank was calculated from the concentrations measured (μg litre^{-1}) at different intervals of incubation period. These residue values were subjected to regression analysis, and were found to fit well into eqns (1) to (3) and the half-life data are given in Table 3. The actual amount of tebufenozide (μg) in each of the three sediment layers was calculated from the concentrations (μg kg^{-1}) measured, added together and multiplied by the total number of vials (40) placed in each aquarium tank to compute the entire amount of tebufenozide present in the sediment in each tank (Table 4). The total residues in the water and sediment were added together to obtain the total residue (μg) and percentage of residues in the entire tank. The results show a gradual decline from 100% on day zero to about 47% on the 90th day (Table 4). Values of the total residues in each tank were fitted into eqns (1) to (3) to calculate the DT_{50} (Table 3). The DT_{50} values for the total residue in water and for the total residue in the entire tank were similar (67 and 72 days respectively). Thus, the water concentrations seemed to have contributed more to the overall rate of disappearance of residues than the sediment concentrations. However, further investigations are needed on dissipation rates in each sediment layer, before any definite conclusion can be drawn on these aspects.

3.2 Study II: Behaviour of tebufenozide in aquatic enclosures under field conditions

3.2.1 Distribution of tebufenozide in surface microlayer

Tebufenozide concentrations in samples of surface water collected from the enclosures using the GFF discs at different intervals of time after treatment are given in Table 5. Each value represents the mean of 12 GFF discs (four discs per enclosure × three enclosures per dosage). Pre-spray and control samples did not contain any tebufenozide. Initial (1 h post-spray) amounts in the surface microlayer were about 11 to 18 times higher than the expected equilibrium value (0·0005 to 0·005 mg cm^{-2}) and ranged from 0·0089 to 0·0562 mg cm^{-2}. This high variability showed incomplete mixing of tebufenozide in the water column at 1 h post-spray. However, surface concentrations decreased

TABLE 4
Study I—Model II: Disappearance of Tebufenozide from Water and Movement into Sediment at 15°C in an Environmental Chamber

	Residue levels of tebufenozide							
Incubation time (days)	*Water (0–10 cm from the top) (μg litre^{-1})*	*Total amount in water (μg)*	*Sediment (top 2 cm) (μg kg^{-1}) (μg)[a]*	*Sediment (middle 2 cm) (μg kg^{-1}) (μg)[a]*	*Sediment (bottom 3 cm) (μg kg^{-1}) (μg)[a]*	*Total amount in sediment (μg)[b]*	*Total residue (μg)[c]*	*Total residue (%)*
0	350	14000	ND[d]	ND	ND	ND	14000	100
1	332	13280	873 (9·95)	13·46 (0·153)	ND	404 (10·11)	13684	97·7
2	329	13160	1072 (12·22)	21·68 (0·247)	ND	499 (12·47)	13659	97·6
4	317	12680	1671 (19·05)	29·20 (0·333)	ND	775 (19·38)	13455	96·1
7	298	11920	2095 (23·88)	33·42 (0·381)	ND	971 (24·26)	12891	92·1
10	280	11200	2894 (32·99)	37·70 (0·430)	ND	1337 (33·42)	12537	89·5
15	261	10440	3444 (39·26)	41·00 (0·467)	ND	1589 (39·73)	12029	85·9
20	242	9680	3270 (37·28)	39·94 (0·455)	ND	1509 (37·73)	11189	79·9
25	225	9000	2898 (33·04)	26·26 (0·413)	ND	1338 (33·45)	10338	73·8
30	199	7960	2685 (30·61)	32·66 (0·372)	ND	1239 (30·98)	9199	65·7
35	185	7400	2315 (26·39)	31·34 (0·357)	ND	1070 (26·75)	8470	60·5
45	171	6840	2035 (23·20)	26·90 (0·307)	ND	940 (23·51)	7780	55·6
55	160	6400	1895 (21·60)	20·40 (0·233)	ND	873 (21·84)	7273	52·0
70	153	6120	1775 (20·24)	16·06 (0·183)	ND	817 (20·42)	6937	49·5
90	145	5800	1656 (18·88)	13·24 (0·151)	ND	761 (19·03)	6561	46·9

[a,b,c] See footnote to Table 2.
[d] ND = not detectable; limit of detection, 0·02 μg kg^{-1}.

rapidly between 1 and 8 h after application due to rapid mixing of the chemical with the water in the enclosures. At 8 h post-spray, the insecticide had mixed well and attained concentrations of 0·0008, 0·0011, 0·0030 and 0·0056 mg cm^{-2} (Table 5) in the four enclosures, which were close to the nominal concentrations at 24 h.

3.2.2 *Vertical mobility of tebufenozide in water*

The concentrations (mg litre^{-1}) of tebufenozide in water at 0·5, 1·5 and 2·5 m depths in the enclosures at 1, 4, 8 and 24 h post-spray are given in Table 6. At the lower dosage (0·05 mg litre^{-1}), the mixing of the chemical in the water column was rapid, and the equilibrium concentration (0·071 to 0·076 mg litre^{-1}) was attained within 1 h post-spray. In contrast, such rapid mixing did not occur at the higher dosage (0·26 mg litre^{-1}), because the concentration (0·207 mg litre^{-1}) at the 2·5 m depth was about 62% of the concentration (0·344 mg litre^{-1}) found at the 0·5 m depth. At 4 h post-spray, the concentration levels were nearly the same at all three depths, a finding in agreement with those made earlier at 8 h post-spray on the mixing patterns of tebufenozide in the surface microlayer (Table 5). Thus, the mixing patterns of the chemical in Study II were different from those observed in Study I, presumably because the two ecosystems were basically different. The material was applied onto the water surface in

TABLE 5
Study II: Dissipation of Tebufenozide from Microlayer of Water Surface in Aquatic Enclosures

Time (h) after application	*Concn of tebufenozide in water (mg cm^{-2}) (±SD)[a] at the four dosages applied (mg litre^{-1})*			
	0·05	*0·10*	*0·26*	*0·50*
1	0·0089 (±0·0008)	0·0109 (±0·0018)	0·0324 (±0·0273)	0·0562 (±0·0310)
4	0·0019 (±0·0007)	0·0022 (±0·0013)	0·0042 (±0·0020)	0·0103 (±0·0037)
8	0·0008 (±0·0002)	0·0011 (±0·0005)	0·0030 (±0·0020)	0·0056 (±0·0019)
24	0·0005 (±0·0001)	0·0009 (±0·0003)	0·0020 (±0·0005)	0·0042 (±0·0004)

[a] Each value represents the mean of 12 measurements.

TABLE 6
Study II: Vertical Mobility of Tebufenozide in Aquatic Enclosures

Time (h) after application	*Concentration of tebufenozide in water (mg litre^{-1}) (±SD)*[a]					
	Dosage applied 0·05 mg litre^{-1} Depth (m)			*Dosage applied 0·26 mg litre^{-1} Depth (m)*		
	0·5	*1·5*	*2·5*	*0·5*	*1·5*	*2·5*
1	0·073 (±0·008)	0·074 (±0·011)	0·073 (±0·008)	0·334 (±0·101)	0·335 (±0·039)	0·207 (±0·045)
4	0·073 (±0·007)	0·074 (±0·010)	0·073 (±0·019)	0·364 (±0·062)	0·367 (±0·055)	0·365 (±0·040)
8	0·072 (±0·010)	0·075 (±0·007)	0·076 (±0·010)	0·360 (±0·033)	0·357 (±0·033)	0·345 (±0·014)
24	0·071 (±0·011)	0·072 (±0·011)	0·072 (±0·009)	0·323 (±0·029)	0·324 (±0·030)	0·322 (±0·020)

[a] Each value represents the mean of 12 measurements.

the field study, whereas it was uniformly pre-mixed with water in the laboratory study (Model II).

3.2.3 *Persistence of tebufenozide in water*

The residual concentration levels measured up to 393 days post-spray indicate that, except at the highest dosage of 0·50 mg litre^{-1}, the overall dissipation of tebufenozide at the three lower dosages was low and gradual (Table 7). At 21 days post-spray, only about 26, 25 and 33% of the initial concentration levels disappeared from water in the three enclosures that received the three lowest dosages. In contrast, nearly 51% of the material disappeared during the same period from water in the enclosure treated with the highest dosage. This unusually high rate of dissipation was primarily due to the low solubility of tebufenozide (0·83 μg ml^{-1} at 20°C), resulting in precipitation of the material from water, followed by adsorption and accumulation in the sediment, especially when the water temperature dropped to about 11°C (Table 1). On the last day of sampling prior to lake freeze-up (154 days post-spray), the average concentrations in the enclosures ranged from 6 to 10% of the initial values. Prior to the termination of the study in the summer of 1993 (393 days post-spray) about 2–5% of the initial concentrations were still detected in the water, indicating that the material had over-wintered and persisted in water in spite of the various degradative processes (physicochemical and biological) acting on the chemical.

Pesticides, following their release in the environment, are gradually lost from the different substrates due to hydrolysis, oxidation, volatilization, photolysis, uptake by biota (algae, invertebrates and aquatic plants were present in the enclosures but not fish and other higher organisms), adsorption to sediment and microbial action. Because of the low vapour pressure and high melting point,[4] volatilization might not have played a major role in the dissipation of tebufenozide. Similarly, hydrolysis could not have played an important role, since the chemical has been found to be appreciably stable in water at different pH values.[11] Co-distillation from the surface microlayer into the ambient air is a likely pathway. However, photolysis[11] is more likely because the water in the enclosures was clear [low turbidity (Table 1)] during the summer months and therefore, sunlight could have reached deeper levels in the enclosures. Nonetheless, adsorption to the sediment appears to be the major pathway of removal of the chemical from water. Since tebufenozide has a low water solubility (0·83 μg ml^{-1} at 20°C) and a high octanol/water partition coefficient (K_{ow} 2·0 × 10^4)[4], it would be adsorbed onto the lipophilic sediment particles. Furthermore, our laboratory microcosm study[11] showed that degradation of tebufenozide proceeded more slowly in autoclaved lake water than in unautoclaved lake water, suggesting that microbial activity could have played an important role in the degradation of the chemical in aquatic enclosures.

The data in Table 7 were subjected to regression analysis, and were found to fit into eqns (1) to (3) (Table 8). The high DT_{50} values (ranging from about 76 to 81 days) suggest that tebufenozide will likely persist in natural waters, and could even over-winter as a result of slow metabolism during the cold winter season. While the long persistence of tebufenozide may be considered undesirable, the biological consequences of such a long persistence should be taken into account. Studies conducted so far by other researchers in our laboratory have indicated minimal impact of the chemical on aquatic organisms (Kreutzweiser, pers. commun.).

It is also important to mention that the observed long persistence could be due to the type of ecosystem used in this study. While the present findings may represent the 'worst case scenario' wherein large quantities of tebufenozide were applied directly onto water bodies,

TABLE 7
Study II: Mean Concentration Levels of Tebufenozide in Water Samples from Aquatic Enclosures After Application at Four Dosages

Time (days) after application	*Concn of tebufenozide in water (mg litre^{-1}) (±SD)[a] at the four dosages applied (mg litre^{-1})*			
	0·05	*0·10*	*0·26*	*0·50*
0·33	0·074 (±0·010)	0·133 (±0·009)	0·331 (±0·021)	0·659 (±0·034)
1	0·072 (±0·012)	0·124 (±0·007)	0·330 (±0·003)	0·625 (±0·041)
2	0·069 (±0·010)	0·121 (±0·006)	0·309 (±0·007)	0·579 (±0·035)
3	0·066 (±0·009)	0·119 (±0·005)	0·304 (±0·005)	0·547 (±0·020)
5	0·060 (±0·007)	0·112 (±0·001)	0·285 (±0·011)	0·527 (±0·007)
8	0·060 (±0·006)	0·110 (±0·002)	0·261 (±0·016)	0·500 (±0·010)
12	0·056 (±0·007)	0·103 (±0·003)	0·232 (±0·012)	0·363 (±0·021)
16	0·056 (±0·006)	0·103 (±0·003)	0·225 (±0·010)	0·343 (±0·026)
21	0·055 (±0·006)	0·100 (±0·005)	0·223 (±0·010)	0·323 (±0·025)
28	0·051 (±0·005)	0·101 (±0·001)	0·210 (±0·018)	0·319 (±0·020)
35	0·049 (±0·003)	0·101 (±0·003)	0·210 (±0·016)	0·311 (±0·015)
49	0·045 (±0·002)	0·090 (±0·001)	0·202 (±0·010)	0·298 (±0·010)
70	0·037 (±0·008)	0·077 (±0·008)	0·204 (±0·001)	0·287 (±0·020)
92	0·030 (±0·009)	0·069 (±0·007)	0·189 (±0·009)	0·293 (±0·011)
119	0·017 (±0·008)	0·037 (±0·012)	0·090 (±0·072)	0·153 (±0·064)
154	0·005 (±0·003)	0·008 (±0·003)	0·033 (±0·028)	0·052 (±0·040)
342	0·005 (±0·002)	0·007 (±0·003)	0·016 (±0·009)	0·018 (±0·006)
355	0·003 (±0·002)	0·006 (±0·004)	0·014 (±0·008)	0·021 (±0·006)
393	0·002 (±0·002)	0·003 (±0·002)	0·012 (±0·006)	0·030 (±0·007)

[a] Values represent means of six measurements for three replicate samples.

such applications lack environmental realism. In the operational spray programs, tebufenozide would be expected to be applied at 70–140 g AI ha^{-1}, and the concentrations reaching water bodies would be substantially lower than those encountered in this study. Under such circumstances, persistence in water, accumulation in the sediment etc., will likely be very different. Therefore, further research is needed to determine the environmental behaviour of tebufenozide under typical conditions of operational spray programs used in forestry for insect control.

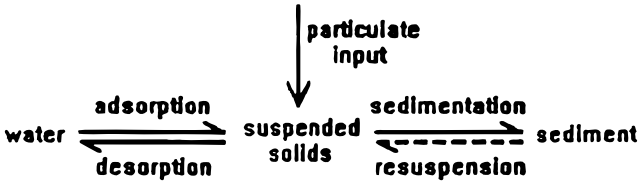
3.2.4 Tebufenozide residues in suspended solids

Adsorption of tebufenozide onto suspended solids (Table 9) appeared to increase with increased dosage. At the dosage of 0·26 mg litre^{-1}, the amount of the analyte in suspended solids was 22·4% at 1 h post-spray and increased to 37·0% at 0·50 mg litre^{-1}. However, these differences could have also been due to variations in the amount of suspended solids among the different enclosures. Nonetheless, over the period of time after treatment, the concentrations in suspended solids from both enclosures decreased gradually and reached almost

TABLE 8
Study II: Dissipation Kinetics of Tebufenozide in Water Using the Equations
$Y = B\,e^{-Ct}$
$\mathrm{Log}_{10}\ Y = \mathrm{Log}_{10}\ B - (C/2.303)t$
$DT_{50} = (2.303\ \log_{10}\ 2)/C$

Dosage (*mg litre*$^{-1}$)	*Rate constant C*	*Coefficient of determination* (R^2)	DT_{50} (*days*)
0·05	−0·0088	0·927	79·2
0·10	−0·0092	0·923	75·8
0·26	−0·0085	0·953	81·2
0·50	−0·0086	0·926	80·7

similar values of about 5–9% beyond 21 days post-spray. This trend was maintained until the last day (393) of sampling, probably due to the equilibrium established among the compartments as shown below:



The turbidity of water (Table 1) in the enclosures was low. Therefore, the number of suspended particles per unit volume of water would have also been low. As a result, adsorption to suspended solids is an unlikely pathway of removal of tebufenozide from the subsurface waters.

3.2.5 Persistence of tebufenozide in sediment

The residues of tebufenozide in sediment samples collected from the four enclosures at various intervals of time (0·33 to 393 days) after spray are given in Table 10. To minimize variability in results, residues were measured on a dry weight basis and were adjusted to the 57% average moisture level of the sediment. The pre-spray and control samples did not contain any residues of tebufenozide.

The initial residues (at 0·33 days post-spray) in the four enclosures increased when the applied dosage increased, and the mean concentrations were 1·00, 1·57, 6·13 and 8·99 mg kg^{-1} at dosages of 0·05, 0·10, 0·26 and 0·50 mg litre^{-1} respectively. Concentrations also increased gradually with time after spray, obviously due to adsorption. Peak levels of 1·55 and 3·05 mg kg^{-1} were observed on day 28 post-spray in enclosures treated at 0·05 and 0·10 mg litre^{-1} respectively (Table 10). On the other hand, peak levels of 11·44 and 26·97 mg kg^{-1} were observed only at 35 days post-spray in enclosures treated at 0·26 and 0·50 mg litre^{-1} respectively. The high standard deviations from the mean values were probably due to temperature fluctuations during the course of the study and the changes in water levels of the enclosures.

After the peak levels, the residue levels decreased gradually until the last day (393) of sampling, except in the enclosures treated at 0·26 and 0·50 mg litre^{-1}, where the residue levels showed a slight increase on the 154th day and decreased afterwards (Table 10). This increase was due to the precipitation and adsorption of the chemical when the water started to freeze on the 154th day post-spray. On the last day of the investigation (393 days post-spray) the mean concentrations (mg kg^{-1}) in enclosures treated at 0·05, 0·10, 0·26 and 0·50 mg litre^{-1} (the maximum operational concentrations ranging from 0·05 and 0·10 mg litre^{-1}), were 0·25, 0·39, 2·77 and 13.20 respectively. These values corresponded to 25·0, 24·8, 45·2 and 146·8% of the initial (0·33 days post-spray) concentrations in sediments in the respective enclosures. Because of the initial increase

TABLE 9
Study II: Percentage Concentrations of Tebufenozide in Water and Suspended Solids After Filtering Through a 5-μm Filter

	Dosage applied (*mg litre*$^{-1}$)			
	0·26		*0·50*	
Time (*days*) *after application*	*Concn in water* (*measured*)[a] (%)	*Concn in suspended solids* (*calc.*) (%)	*Concn in water* (*measured*)[a] (%)	*Concn in suspended solids* (*calc.*) (%)
1	77·6	22·4	63·0	37·0
5	91·2	8·8	71·8	28·2
21	92·0	8·0	90·0	10·0
49	94·5	5·6	92·3	7·7
92	94·8	5·2	92·0	8·0
119	93·8	6·2	91·4	8·6
393	95·3	4·7	92·5	7·5

[a] For initial concentration of tebufenozide in non-filtered water, see Table 7.

TABLE 10
Study II: Mean Concentration Levels of Tebufenozide in Sediment Samples (wet weight) from Aquatic Enclosures After Application at Four Dosages

Time (days) after application	*Concn of tebufenozide in sediment (mg kg^{-1}) (±SD)[a] at the four dosages applied (mg litre^{-1})*			
	0·05	*0·10*	*0·26*	*0·50*
0·33	1·00[a] (±0·35)[b]	1·57 (±0·22)	6·13 (±1·17)	8·99 (±0·94)
1	1·13 (±0·39)	2·68 (±2·03)	6·32 (±0·57)	11·55 (±1·92)
2	1·16 (±0·48)	2·02 (±0·47)	6·98 (±0·48)	14·57 (±1·08)
3	1·23 (±0·43)	2·42 (±0·81)	5·71 (±1·18)	16·53 (±2·16)
5	1·30 (±0·34)	3·10 (±0·19)	5·27 (±2·35)	21·32 (±4·44)
8	1·32 (±0·67)	2·70 (±0·49)	7·27 (±3·11)	19·19 (±5·62)
12	1·32 (±0·89)	2·73 (±0·36)	7·68 (±2·11)	21·09 (±9·73)
16	1·43 (±1·13)	2·95 (±0·56)	7·17 (±0·79)	23·34 (±8·67)
21	1·47 (±1·02)	2·78 (±0·84)	11·06 (±3·22)	19·30 (±11·35)
28	1·55 (±0·82)	3·05 (±0·96)	11·23 (±4·58)	26·38 (±9·44)
35	1·47 (±0·66)	2·97 (±0·89)	11·44 (±7·36)	26·97 (±9·22)
49	1·55 (±0·72)	2·41 (±1·36)	8·03 (±3·80)	23·40 (±8·42)
70	1·23 (±0·60)	2·42 (±1·58)	4·17 (±1·76)	23·84 (±14·83)
92	0·92 (±0·27)	1·90 (±0·87)	4·19 (±1·42)	22·16 (±9·81)
119	0·70 (±0·30)	2·09 (±0·34)	3·99 (±0·45)	21·30 (±10·01)
154	0·58 (±0·34)	0·85 (±0·19)	4·46 (±1·65)	28·13 (±12·12)
342	0·24 (±0·12)	0·43 (±0·20)	3·39 (±1·20)	19·80 (±5·81)
355	0·27 (±0·08)	0·43 (±0·18)	3·01 (±1·80)	14·40 (±6·21)
393	0·25 (±0·11)	0·39 (±0·19)	2·77 (±0·98)	13·20 (±4·93)

[a] Values represent the mean of 6 measurements for 3 replicate samples.

in residues and attainment of peak levels, DT_{50} values were not calculated for the sediments.

The present investigation clearly demonstrated that lake sediment adsorbed tebufenozide and acted as an effective sink. The chemical persisted over the winter months, despite the possibility of microbial activity. The high adsorptive power of the sediment was due to its large surface area, high organic carbon content (Table 1), and lipophilicity. The high K_{ow} of tebufenozide favoured its solubility in lipophilic materials such as the sediment in the enclosures. While the accumulation of the chemical in the sediment may be of some concern, its prolonged persistence is ultimately important. The degradation kinetics of the bound residues, together with bioavailability, toxicity and bioaccumulation of the parent material and metabolites to sediment-dwelling organisms, must be taken into account before determining the acceptability of the chemical for large-scale broadcast applications for forest insect control.

4 CONCLUSIONS

The present investigation indicated that tebufenozide persisted for a considerable length of time in aquatic

ecosystems under both laboratory and field conditions. The laboratory study, conducted under conditions of constant temperature, water pH and continuous photoperiod using two aquatic models set up in glass aquaria tanks, indicated that the chemical moved readily from treated water into sediment and was adsorbed there. Vertical downward movement of the adsorbed material from the top layer of sediment into layers beneath did not occur readily. Also, very little release of the adsorbed material into water occurred. However, the concentration of chemical in both substrates (i.e. water and sediment) decreased gradually during the 90-day incubation period. The chemical disappeared from the top 2-cm layer of the sediment with a half-life of 64 days. However, it disappeared from the middle 2-cm and bottom 3-cm layers at a slower rate, with half-lives of disappearance of >90 days. The half-life of disappearance from water was 67 days.

The field microcosm study, conducted in aquatic enclosures under fluctuating conditions of temperature, water pH (Table 1) and photoperiod, also showed that the chemical moved downwards from the applied location (top surface) and was adsorbed onto sediment. Nonetheless, the chemical persisted for a long time (393 days post-spray) both in water and in sediment, suggesting an over-wintering potential of tebufenozide. Persistence in sediment was even longer under field conditions than under laboratory conditions. At 90 days post-treatment, only about 55% of the applied material persisted in sediment (37, 54·5 and 73·7% in the top, middle and bottom layers respectively, with an average of 55%) in laboratory models, indicating little accumulation. In the field study, the material not only persisted but also was accumulated in the sediment, because at 92 days post-spray the residues were about 24·5 times higher (average 18·4, 19·0, 16·1 and 44·3 times in enclosures treated at 0·05, 0·10, 0·26 and 0·50 mg litre^{-1} respectively) than the applied concentration levels. Similar to the finding in sediment, residues in water also decreased more rapidly in the laboratory study than in the field study, because the concentration level (145 μg litre^{-1}) at 90 days post-treatment was about 41·4% of the applied value of 350 μg litre^{-1}. In the field study, however, about 65% of the applied tebufenozide persisted in water at 92 days post-spray (average of 60, 69, 73 and 59% in enclosures treated at 0·05, 0·10, 0·26 and 0·50 mg litre^{-1} respectively). The longer persistence observed under field conditions was probably due to the fluctuating temperature, water pH and photoperiod encountered, compared to the constant environmental conditions and water pH utilized in the laboratory study. The continuous photoperiod especially would be expected to increase the rate of disappearance, since tebufenozide is known to be susceptible to photodegradation.[11]

While the long persistence of tebufenozide in the environmental substrates is partly attributable to its molecular, structural and physicochemical properties,[1,12] the type of ecosystem used in this study may also contribute to long persistence. The enclosures represent the 'worst case scenario' wherein tebufenozide was applied directly onto water bodies. Under operational use patterns, however, the material would be sprayed aerially, and the concentrations reaching water bodies would be substantially lower. Under such circumstances, persistence in water, accumulation in the sediment, etc., will likely be different. Therefore, further field studies on environmental fate and persistence under realistic situations are needed using naturally occurring aquatic ecosystems (e.g. ponds and streams), before any definite conclusion can be made on the acceptability of the chemical for large-scale broadcast applications in forestry

ACKNOWLEDGEMENTS

The present investigation was jointly funded by Natural Resources Canada (NRCan), Canadian Forest Service (CFS) and Rohm and Haas Company, Westhill, Ontario, Canada. The author thanks D. Kreutzweiser for applying the formulation of tebufenozide over aquatic enclosures; D. Kurylo and J. Ramakers (NRCan, CFS) for providing data on water quality and sediment texture; R. Nott, J. Curry, L. Sloane, J. W. Leung, J. Zhu and the summer students who provided excellent technical assistance in the laboratory and field studies; and D. R. Hawkins, R. Deakyne and C. Patel of Rohm and Haas Company for their interest in this research.

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